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Synthetic mammalian gene circuits for biomedical applications

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Synthetic biology is the science of reassembling cataloged and standardized biological items in a systematic and rational manner to create and engineer functional biological designer devices, systems and organisms with novel and useful, preferably therapeutic functions. Synthetic biology has significantly advanced the design of complex genetic networks that can reprogram metabolic activities in mammalian cells and provide novel therapeutic strategies for future gene-based and cell-based therapies. Synthetic biology-inspired therapeutic strategies provide new opportunities for improving human health in the 21st century. This review covers the most recent synthetic mammalian circuits designed for therapy of diseases such as metabolic disorders, cancer, and immune disorders. We conclude by discussing current challenges and future perspectives for biomedical applications of synthetic mammalian gene networks.

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Introduction

Synthetic biology is the science of designing and creating novel functional devices, systems, and new organisms by applying engineering principles [1–3]. In the early stages of synthetic biology, many synthetic devices were designed and constructed in prokaryotes or lower eukaryotes, including toggle switches [4], oscillators [5–7], timers [8], counters [9], clocks [10], pattern detectors [11], band-pass filters [12], and intercellular communication systems [13,14]. Pioneering experiments later confirmed the therapeutic possibilities of synthetic biology. These experiments included designing bacteriophage to switch off the bacterial SOS DNA repair system [15], engineering bacteria or viruses for cancer-targeting therapy [16–21], engineering *Escherichia coli* to prevent cholera infection [22], and engineering yeast cells to produce the precursor of the antimalarial drug artemisinic acid [23]. Although synthetic devices can easily be engineered and programmed in prokaryotic systems, the clinical application of prokaryotic synthetic circuits is limited. Recent studies have therefore focused on designing heterologous transcription-control systems in mammalian cells. This research opens a door for developing new therapeutic strategies, especially gene-based and cell-based therapies, to treat human diseases [24,25•,26–28] (Figure 1). This review focuses on the latest in mammalian synthetic biology, including designing state-of-the-art, synthetic gene networks and developing prototype treatment strategies.

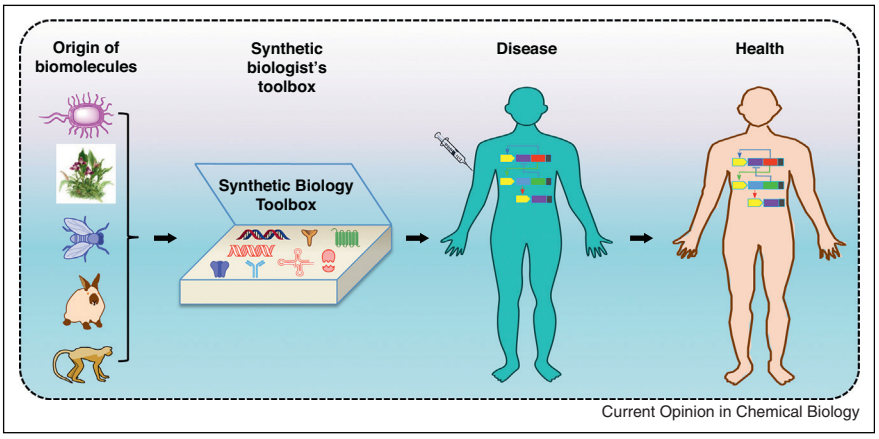
Synthetic biology therapeutic strategies for metabolic disorders

Type 2 diabetes is a metabolic disorder characterized by high blood glucose as a result of insulin resistance or relative insulin deficiency [29]. It is common both in developed and developing countries and approximately 6 percent of the world's population is affected by it. Traditional therapeutic strategies include a strict diet and increased exercise, daily insulin injections, or medication before every meal. These strategies combine high cost with low patient convenience.

Two highly promising, synthetic biology-based therapeutic strategies for treating diabetes were recently demonstrated. The first example is the construction of an optogenetic device to control blood glucose homeostasis [30••]. Using light as a traceless, molecule-free, input signal to trigger transgene expression in living organisms is becoming popular [31–35]. Ye *et al.* engineered expression of shGLP-1, a hormone that can restore blood glucose homeostasis in type 2 diabetic mice, to be under the control of blue light. Melanopsin, a blue-light sensor protein, is ectopically expressed in HEK-293 cells, and when triggered by the presence of blue light, activates the nuclear factor of activated T cells (NFAT) by phosphorylation via an intracellular signaling cascade. This cascade results in HEK-293 cells expressing shGLP-1, which has been placed under the NFAT-controlled promoter. These engineered cells were then encapsulated and implanted into a mouse model of human type 2 diabetes. Mice illuminated by blue light showed enhanced blood-glucose homeostasis (Figure 2a). Another study later confirmed that diabetes treatment by light-triggered GLP-1 expression was possible [36].

Stanley *et al.* [37••] developed an insulin expression system under the control of radio waves to restore glucose

Figure 1



Synthetic gene circuits designed for treating human disease. Biologists capitalize on natural biomolecules from various organisms (including microbes, plants, and mammals), which are well understood and have been biochemically studied. The synthetic biology toolbox consists of those well-studied, high-value biomolecules that consist of functional DNA, RNA, and proteins. By applying engineering principles, synthetic biologists reassemble and standardize these basic toolbox components in a rational way to create and engineer functional therapeutic gene networks. These networks are then uploaded into encapsulated cell implants, which are placed into the body and regulated to produce valuable therapeutic biomolecules. These biomolecules treat the disease, and bring the patient back to health.

homeostasis in diabetic mice. In this system, a temperature-sensitive channel, TRPV1, is antibody-coated with oxide nanoparticles, and therefore triggers calcium influx when heated by radio waves. This influx of calcium stimulates both the expression and release of insulin, from a bioengineered insulin construct driven by a calcium-sensitive promoter. When the mice harboring tumor xenografts expressing insulin are exposed to radio waves, the system is activated to express and release insulin from the tumors and lower blood glucose (Figure 2b).

Metabolic syndrome, a prime 21st-century epidemic, is a combination of disorders and risk factors, including hypertension, hyperglycemia and dyslipidemia, all of which collectively increase the risk of cardiovascular disease [38,39]. The traditional therapeutic strategy is to identify and treat each risk factor separately [40]. However, this single-risk-factor treatment strategy can cause polypharmacy which represents a lifestyle disincentive for patients and may accumulate side effects [40]. Recently, a multifunctional synthetic circuit for simultaneously treating multiple risk factors of metabolic syndrome was developed [41••]. In this circuit, the signal transduction of the chimeric trace-amine-associated receptor 1 (cTAAR1), triggered by the antihypertensive drug guanabenz (Wytensin®), is functionally rewired to use cAMP and cAMP-dependent PKA. This then activates the cAMP-response element binding protein (CREB1), which binds to the CREB1-specific promoter driving the therapeutic peptide hormone GLP-1-Fc-Leptin. This system has been successfully engineered and implanted into mice developing metabolic syndrome symptoms and simultaneously attenuate hypertension, hyperglycemia, and dyslipidemia in mice (Figure 2c). This combination of classic and synthetic biology-based treatments may improve treatment success and provide new therapeutic strategies for multifactorial diseases.

A more advanced synthetic biology-derived prosthetic network would be able to sense and monitor host metabolic parameters and respond accordingly without further input. An example of this type of sensor-effector prosthetic network was designed to sense the uric acid concentrations in the blood, and control the expression of a urate oxidase (smUOX), which acts to restore urate homeostasis. This could be a treatment strategy for gout and tumor lysis syndrome [42] (Figure 2d). In this circuit, the smUOX expression level is controlled by the bacterial HucR repressor, which has been fused with the transcription-silencing domain KRAB (HucR-KRAB). Upon binding to the cognate operator site, HucR-KRAB inhibits smUOX production. In the presence of urate, the HucR-KRAB is released from the operator site, which initiates the expression of smUOX. Engineered cells containing this prosthetic uric-acid-responsive transcription network were implanted into acute hyperuricaemic mice. This network was able to dissolve uric-acid crystal deposits in the kidneys, proving that this concept works to ameliorate disease symptoms.

Synthetic logic circuits for cancer therapy

Cancer is a large group of diseases involving rapid, uncontrollable creation of abnormal cells that can invade nearby healthy tissues and spread to other organs. One of the key challenges for cancer therapy is to eliminate